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Published in:
Biomicrofluidics

Link to article, DOI:
10.1063/1.4905093

Publication date:
2014

Document Version
Publisher's PDF, also known as Version of record

Protein and cell patterning in closed polymer channels by photoimmobilizing proteins on photografted poly(ethylene glycol) diacrylate

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(Received 8 October 2014; accepted 16 December 2014; published online 30 December 2014)

Definable surface chemistry is essential for many applications of microfluidic polymer systems. However, small cross-section channels with a high surface to volume ratio enhance passive adsorption of molecules that depletes active molecules in solution and contaminates the channel surface. Here, we present a one-step photochemical process to coat the inner surfaces of closed microfluidic channels with a nanometer thick layer of poly(ethylene glycol) (PEG), well known to strongly reduce non-specific adsorption, using only commercially available reagents in an aqueous environment. The coating consists of PEG diacrylate (PEGDA) covalently grafted to polymer surfaces via UV light activation of the water soluble photoinitiator benzoyl benzylamine, a benzophenone derivative. The PEGDA coating was shown to efficiently limit the adsorption of antibodies and other proteins to <5% of the adsorbed amount on uncoated polymer surfaces. The coating could also efficiently suppress the adhesion of mammalian cells as demonstrated using the HT-29 cancer cell line. In a subsequent equivalent process step, protein in aqueous solution could be anchored onto the PEGDA coating in spatially defined patterns with a resolution of $<15\ \mu$m using an inverted microscope as a projection lithography system. Surface patterns of the cell binding protein fibronectin were photochemically defined inside a closed microfluidic device that was initially homogeneously coated by PEGDA. The resulting fibronectin patterns were shown to greatly improve cell adhesion compared to unexposed areas. This method opens for easy surface modification of closed microfluidic systems through combining a low protein binding PEG-based coating with spatially defined protein patterns of interest. © *2014 AIP Publishing LLC*. [http://dx.doi.org/10.1063/1.4905093]

I. INTRODUCTION

Microfluidic polymer devices are used in a wide range of applications including (bio)sensing, precise mixing, and cell culture.[1–5] The small channel cross-section of microfluidic channels enables manipulation and analysis of minute sample volumes. However, the increasing surface to volume ratio with decreasing channel cross-section enhances passive adsorption of molecules from solution, which depletes the solute and contaminates the channel surfaces.[6–10] Passive non-specific adsorption to polymer surfaces can be reduced or prevented in different ways, for example, by saturating (blocking) the surface by a non-interfering molecule or by the use of materials or coatings with inherently low binding of proteins.[11–14] Blocking with other strongly adsorbing proteins at high concentrations is often used, in particular, albumin.[15–18] However, the introduction of a passively adsorbed protein layer is not acceptable in many applications due to requirements of later sterilizability, contamination risks, and the possibility for

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detachment of the physisorbed blocking molecules.[19,20] Physisorbed protein layers are also a poorly defined substrate for subsequent specific protein attachment to be used for application specific analyte or cell interactions. Covalently attached non-protein low protein binding coatings overcome these limitations and are often preferred or required. However, if the surface does not contain readily available reactive groups (e.g., carboxylic acid or amine groups), they have to be introduced before a covalent coating can be applied,[21,22] often calling for multiple process steps that are poorly compatible with production upscaling, in particular, for coatings to be applied inside closed microfluidic channels. Alternatively, the coating can be grown from the surface as previously shown by several groups through the introduction of a low protein binding layer of acrylate-modified poly(ethylene glycol) (PEG) by initiating polymerization of the terminal acrylate group using a radical initiator.[23–28] If only the acrylate groups are activated, a hydrogel is formed in solution without covalent attachment to the surface. The UV light sensitive photoinitiator benzophenone is known from multiple studies to efficiently activate both acrylates and polymer surface groups by radical transfer and hydrogen abstraction, respectively.[29–31] Unfortunately, benzophenone is only soluble in organic solvents that may compromise the integrity of the polymer microfluidic system by swelling or even dissolution. Moreover, the large surplus of organic solvent molecules over PEG-acrylate solute molecules, both with abstractable hydrogens, gives a high risk of coupling solvent instead of solute onto the polymer channel surface.[32] Finally, a thick hydrogel unsuitable for microfluidic channels is often produced as a high concentration of acrylate is used to minimize the latter side reaction.[29,32] To overcome these problems, we present a single-step method for depositing a covalently bound low protein binding PEG-based coating and a subsequent single-step method to covalently attach specific proteins at designated areas on the initially applied coating, all within closed polymer microchannel systems, under light control, and using only commercially available reagents. The low protein binding coating results from UV exposure of an aqueous solution of a PEG diacrylate polymer (PEGDA) in low concentration in the presence of 4-benzoyl benzylamine hydrochloride (Bz), a water soluble benzophenone derivative. The coating produced is very thin with a dry thickness of a few nanometers, thus making minimal changes to the channel geometry, and it is shown to prevent adsorption of proteins and adhesion of cells. In a subsequent UV exposure step, we employ a projection lithography system based on a rebuilt inverted microscope to produce micropatterns of photo-immobilized protein inside the closed PEGDA-coated microfluidic channel. Active patterned capture of cells is enabled by photo-immobilizing fibronectin, a cell adhesion promoting protein, on top of the PEGDA-coating within the sealed channel system.

II. MATERIALS AND METHODS

A. Materials

4-benzoyl benzylamine hydrochloride (Bz) was purchased from Fluorochem (Hadfield, UK). Fluorescein-conjugated bovine serum albumin (fluorescein-BSA), phosphate buffered saline (PBS), anti-Mouse IgG (whole molecule)−alkaline phosphatase (AP) antibody produced in goat (A3562, IgG-AP), anti-Mouse IgG (whole molecule)-peroxidase antibody (A4416), alkaline phosphatase yellow (pNPP) liquid substrate (P7998, AP substrate), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) liquid substrate system (A3219, HRP (horseradish peroxidase) substrate), Dulbecco's Modified Eagle's Medium (D5796, DMEM), fetal bovine serum (FBS), and trypsin were purchased from Sigma-Aldrich (St. Louis, MO). All water used were from a Millipore MilliQ purification system (Boston, MA). Acrylate-PEG-acrylate (PEGDA), 5 kDa (PSB-345) was purchased from Creative PEGWorks (Winston-Salem, NC). Nunc 96-well polystyrene microtiter plates (Cat. No. 260860) were purchased from Fisher Scientific (Roskilde, Denmark). Polyethylene (PE) microfluidic devices, μ-Slide Chemotaxis 3D (70 μm high channel), and μ-Slide VI 0.4 (400 μm high channel), both tissue culture treated with ibiTreat, were purchased from ibidi GmbH (Martinsried, Germany). Alamar Blue was purchased from Invitrogen (Carlsbad, CA).

B. X-ray photoelectron spectroscopy (XPS)

XPS was performed on a K-Alpha spectrometer (Thermo Fisher Scientific, UK) using a 400–μm wide monochromatized AlKα X-ray spot with collection of the emitted photoelectrons at pass energies of 200 eV and 50 eV for survey and high resolution spectra, respectively. Elemental compositions were determined from acquired survey scans using the instrument manufacturer's Avantage software package. The coating thickness was measured on polystyrene substrates and was calculated from the attenuation of polystyrene's 291 eV peak: $d = \lambda \sin(\theta) \cdot \ln(I_0/I)$, where d is the coating thickness, λ is the inelastic mean free path (estimated to be 3 nm), θ is the photoelectron take-off angle (90°), I is the measured intensity from the coated sample, and I_0 is the intensity from an uncoated sample.[33]

C. Coating of polymer surfaces

PEG coating solutions consisted of different concentration of PEGDA and Bz in PBS and were applied onto the polymer substrate surface, followed by UV light exposure for 30 min using a custom built photoreactor with a broad emission maximum from 330 to 380 nm (Philips Cleo S-R fluorescent tubes) at an intensity of 18 mW/cm^2. The exposed samples were flushed 3 times with water and ethanol.

D. Adsorption assay with IgG-HRP

IgG-HRP (1 μg/ml in PBS) was added to 96 well polystyrene microtiter plates that were coated with different combinations of PEGDA and Bz in PBS as described above. After 1 h, each surface was washed with PBS three times, and 100 μl of HRP liquid substrate was added. The color change was measured at 2 min intervals using a plate reader (Victor3, Perkin–Elmer, MA). The rate in change in color was converted to surface concentrations using a standard curve made with known IgG-HRP concentrations in solution.

E. Alkaline phosphatase conjugation

A PEGDA coating was made as described above with 1 mg/ml of PEGDA and 0.25 mg/ml of Bz in PBS in a 96 well polystyrene microtiter plate. IgG-AP and Bz dissolved in PBS (concentrations specified in Sec. III) were added to the wells and exposed to UV light for 30 min. Wells were washed with PBS + Tween 20 (0.05%) 3 times and PBS 4 times, and 100 μl of AP substrate was added to each well. The color change was measured with 2 min interval using a plate reader. The rate in change in color was converted to surface concentrations using a standard curve made with known IgG-AP concentrations in solution.

F. Projection lithography system

A Zeiss Axiovert 35M inverted microscope (Carl Zeiss, Oberkochen, Germany) equipped with Zeiss objectives was converted to a projection lithography system by exchanging the field diaphragm of the Köhler illumination system with a holder for Ø20 mm chrome-on-glass shadow masks.[34] The microscope was equipped with a 100 W mercury arc-discharge lamp and a 365 ± 5 nm bandpass filter. The PEGDA-coated polystyrene samples were exposed through a Zeiss 20× Neofluar objective (NA 0.5) or a Zeiss 5× Fluar objective (NA 0.25) that reproduced an image of the mask motif demagnified by a factor of 13.0 or 3.2, respectively, with an irradiance of 720 mW/cm^2 or 70 mW/cm^2, respectively, measured at 365 nm using a calibrated OAI 306 power meter (OAI Instruments, San Jose, CA) with a 365 nm probe. Shadow masks were fabricated by standard photolithography and lift-off of 100 nm chromium on 500 μm borosilicate glass and cut out using a high-intensity 355 nm laser.

G. Patterned protein conjugation

A microfluidic device with 70 μm high channels (ibidi μ-Slide Chemotaxis 3D) was coated with PEGDA as described under Sec. II C using 5 mg/ml PEGDA and 1 mg/ml Bz in PBS with UV illumination for 30 min. Bz (100 μg/ml) and fluorescein-BSA (100 μg/ml) in PBS were added to the channels, and the channel bottom was illuminated by patterned UV light in the projection lithography system. The illuminated surfaces were subsequently washed with water, and the attached protein was visualized on a confocal fluorescence microscope (LSM 5, Carl Zeiss) using illumination at 488 nm and collection of emitted fluorescence at wavelengths $\geq$505 nm.

H. Cell adhesion studies

HT-29 colon adenocarcinoma cells were cultured in DMEM supplemented with 10% v/v FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cell cultures were incubated in a 5% CO_2 humidified incubator at 37 °C. The cells were detached using trypsin/EDTA, followed by centrifugation for 2 min at 1000 rpm and washing of the cell pellet with PBS three times. The cell pellet was resuspended in medium containing 1% v/v or 10% v/v FBS depending on the specific experiment and added to the wells of microtiter plates or to the microfluidic channel system for incubation. Cell viability was measured by adding alamar Blue reagent (10% v/v) to each well, incubating for 4 h, and measuring the fluorescence intensity using a plate reader according to the assay manufacturer's instructions. The results are reported as the difference in fluorescence intensity of the samples and a reference well with alamar Blue reagent incubated for the same time period. Microfluidic channels (μ-Slide VI 0.4) were first coated with PEGDA as described by loading the channel with 5 mg/ml PEGDA and 1 mg/ml Bz in PBS and illuminating with UV light for 2 h. Patterned protein conjugation within the channel proceeded as described in Sec. II G using 100 μg/ml fibronectin and 1 mg/ml Bz in PBS and exposing for 20 min through a 5× objective. Control samples using channel surfaces with or without PEGDA coating were made under identical conditions but without UV exposure during incubation with fibronectin. The treated samples were washed with water and sterilized by exposure to absolute ethanol for 60 min. Each channel was loaded with 50 μl of 10^7 HT-29 cells/ml suspended in DMEM with 1% v/v FBS and incubated for 16 h, before gentle washing with PBS to remove unbound or loosely attached cells. Phase contrast micrographs were recorded on a Zeiss Axiovert 25 microscope equipped with a Sony Exwave HAD digital video camera.

III. RESULTS

A. Nanometer thick PEGDA coatings strongly inhibit protein and cell attachment

Polymer surfaces (polystyrene) were coated using solutions of the water soluble 4-benzoyl benzylamine (Bz) and 5 kDa PEGDA in aqueous inorganic buffer. The solution was simply dispensed onto the polymer surface and exposed to UV light. Figure 1(a) shows a reaction scheme where the benzophenone moiety in Bz is excited by UV illumination to form a bi-radical that can abstract a proton from any carbon-hydrogen containing molecules on a surface or in solution.[35,36] Additionally, the benzophenone moiety can abstract a proton from the acrylate double bond at either or both ends of the PEGDA molecule to initiate radical polymerization of PEGDA.[11] The activation of both the surface and PEGDA enables covalent coating of the formed PEGDA network to the polymer surface.

Optimization studies on planar polystyrene surfaces using XPS and protein adsorption assay as quantitative assays showed that 5 mg/ml PEGDA + 1 mg/ml Bz created the surface with the most PEG coating (Figure S1 in the supplementary material (Ref. 51)) and the least passive protein adsorption (Figure S2 in Ref. 51). The average coating thickness in the dry state was calculated to be <3 nm from the XPS analysis results.

Cell adhesion on different PEGDA coatings was examined by incubating coated surfaces with HT-29 colon adenocarcinoma cells in culture medium with or without addition of 10% v/v

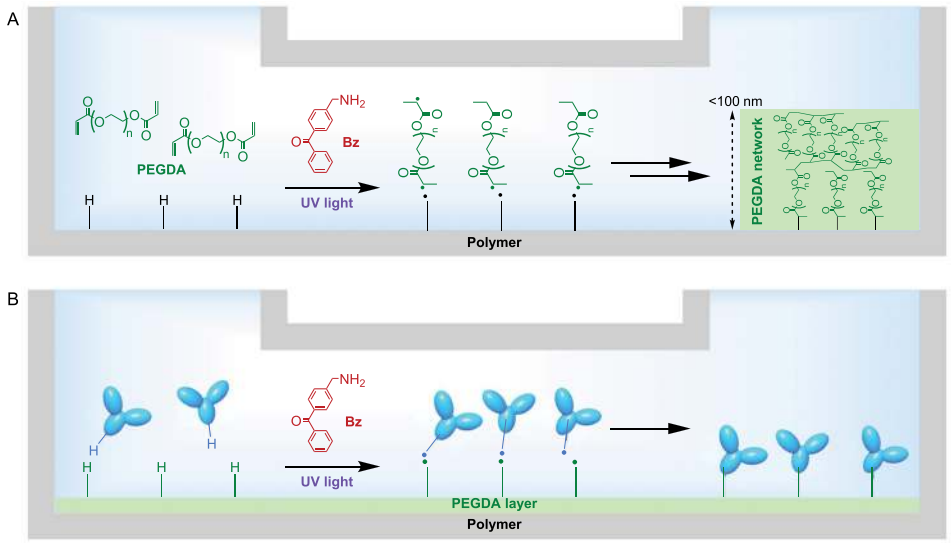

FIG. 1. Equivalent surface chemistry is employed for the initial introduction of a layer with low protein binding and subsequent coupling of functional proteins in aqueous solution inside a closed polymer microchannel. (a) Poly(ethylene glycol) diacrylate (PEGDA) is covalently coupled to the surface and simultaneously polymerized into a nanometer thick (dry state) network by UV illumination in the presence of a water soluble hydrogen-abstracting photoinitiator (Bz). (b) The resulting PEGDA coated microchannel surface may be coated by many types of proteins in a second equivalent reaction step using homogeneous or patterned UV light exposure to guide the spatial position of the coupled proteins.

FBS. After four hours, the surfaces were gently rinsed to remove loosely attached or non-attached cells, and the number of remaining viable cells was quantified using a metabolism assay (alamar Blue, Figure 2). Cells cultured without added serum showed very little adherence to the coated surfaces as revealed by minute fluorescence signals from alamar Blue. Cells cultured with added serum adhered much stronger than without serum. The lowest cell adherence of the serum containing samples was seen on surfaces coated with 5 mg/ml PEGDA + 1 mg/ml Bz consistent with these conditions producing the thickest PEGDA coating and exhibiting the lowest passive protein adsorption (Figures S1 and S2 in Ref. 51).

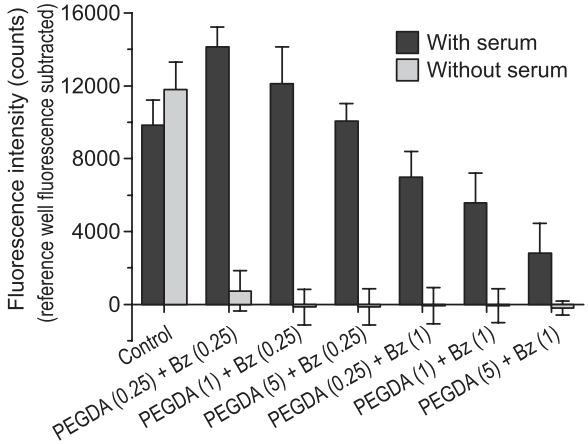

FIG. 2. Adhesion of HT-29 cells on polystyrene surfaces pre-coated with different concentration of PEGDA and Bz (specified in mg/ml). The cells were incubated for 4 h with or without 10% v/v serum. After rinsing of the surfaces, the metabolic activity of the cells was measured with alamar Blue as a measure of the total cell number. The results are reported as the difference in fluorescence intensity of the respective cell loaded wells and a reference cell-free well with alamarBlue incubated for an equal time. The bars and error bars show the mean and standard deviation (n = 3) of the fluorescence intensity caused by the presence of cells in the wells.

B. Active proteins can be photo-immobilized in patterns on PEGDA coatings in closed microchannels

We have previously photo-immobilized proteins onto a PEG coating based on a N-hydroxysuccinimide-conjugated PEG, i.e., without polymerizable acrylate end groups.[37] The photo-immobilization was prepared simply by adding a solution of protein and Bz onto the surface before exposing the surface to UV light. Figure 1(b) illustrates that Bz illuminated by UV light will form a bi-radical that can abstract a proton from any hydrocarbon group on the protein or on the surface. These radicals can then recombine causing covalent bonding of the protein onto the surface. The ability to photo-immobilize proteins on a PEGDA coating was investigated by added an aqueous solution of alkaline phosphatase conjugated IgG (IgG-AP) and Bz to PEGDA-coated polystyrene surfaces and illuminating by UV light for 30 min. The results show that IgG-AP was immobilized onto the PEGDA coated surface under UV illumination (Figure 3(a)) as intended, while non-illuminated PEGDA coated surfaces had almost no immobilized IgG-AP (passively or covalently attached) (Figure 3(b)). Uncoated polymer surfaces showed large non-specific adsorption of IgG-AP with or without UV illumination. These results support that it is possible to photo-immobilize active proteins onto the PEGDA coating.

Patterned attachment of proteins is useful for many advanced applications of microfluidic systems, one example being on-chip cell sorting.[38] Most patterning schemes need direct access to the targeted surfaces which require coating of the channel surfaces prior to sealing of the microfluidic system. Handling of protein coated chip components during assembly calls for extra care, and it strongly limits the available methods for bonding the system as both high temperature and solvents used in many polymer chip bonding schemes may denature the coated proteins irreversibly. Our method overcomes these limitations, as the low protein binding PEGDA coating as well as the covalent coupling of proteins to coated substrates can easily be applied within a closed microfluidic channel, as long as light can penetrate the structure.

For the subsequent patterning of proteins, we developed a simple projection lithography system based on an inverted microscope where a mask motif can be demagnified and projected

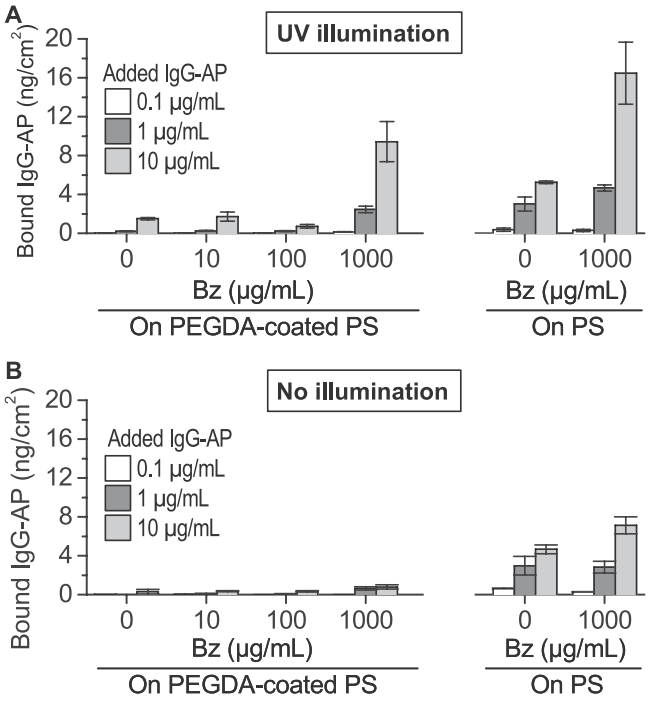

FIG. 3. Photo-immobilization of IgG-AP on polystyrene surfaces with or without pre-coated PEGDA as a function of the IgG-AP and Bz concentrations in solution, and (a) with UV illumination for 30 min or (b) without UV illumination for 30 min. The bars and error bars show the mean and standard deviation (n = 3).

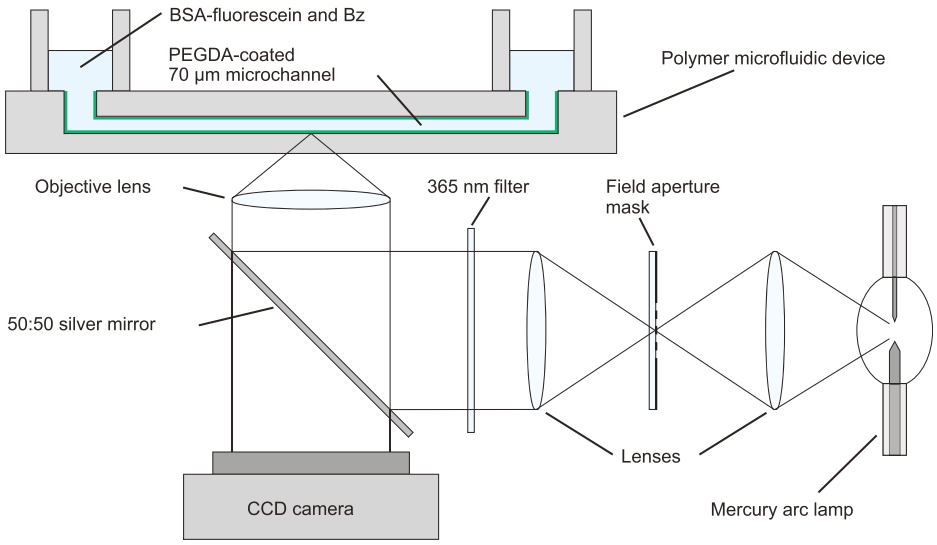

FIG. 4. Sketch of the inverted microscope projection lithography system for photo-patterning of proteins inside a PEGDA-coated closed microfluidic channel. The projection lithography system is based on inserting a chrome-on-glass mask in the field aperture of a Zeiss Axiovert 35M inverted microscope. The mask motif is projected and demagnified onto the inner microchannel walls by the microscope objective lens.

onto an inner channel surface (Figure 4), similar to the system described by Love *et al.*[34] The patterning resolution was evaluated by first coating the inner surfaces of a commercially available microfluidic chip (Figure S3 in Ref. 51) with a homogeneous layer of PEGDA through the addition of aqueous PEGDA and Bz to the channel and flood exposure with UV light. After rinsing the channel, an aqueous solution of fluorescein-labeled BSA and Bz was loaded into the channels, and the bottom channel surface was exposed with patterned UV light defined by the demagnification of a chrome-on-glass shadow mask motif through a 20× objective lens. After rinsing, the exposed surface area was visualized using confocal fluorescence microscopy (Figure 5(a)). The mask motif consisted of arrays of half-pitch lines, where each set of lines is a factor 2/3 smaller than the previous set with widths ranging from 150 μm to 8.8 μm. The demagnified motif projected onto the channel bottom by the optical system had corresponding nominal line widths from 14 μm to 0.82 μm. Fluorescence intensity contrast could be observed for nominal line widths down to 4 μm, while more than 50% contrast between nominally exposed and unexposed areas was measured for nominal line widths $\geq$9.3 μm (Figure 5(b)). As explained in the supplementary material (Sec. II in Ref. 51), the measured material background fluorescence level is subtracted from the raw image data before normalization of the profiles to the measured maximum intensity level. The normalized intensity profiles thus give a measure of the contrast of the photo-immobilized fluorescein-BSA compared to passively adsorbed fluorescein-BSA. The fluorescence intensity from passively adsorbed protein was measured to be $\sim$5% of the maximum intensity. Full-width-at-half-maximum of the nominally 9.3 μm and 14 μm wide lines was measured to be 12 μm and 17 μm, respectively. The uneven peak height is caused by inhomogeneity in the intensity of the light source used for projection lithography as evidenced by direct imaging of the projected light at the sample plane (data not shown). These results show that it is possible to selectively attach a protein inside a closed microfluidic channel with a resolution of $<$15 μm.

We also investigated the possibility for using protein surface patterns on a low protein binding PEGDA background to spatially selective capture and culture mammalian cells. The closed channels of a microfluidic polymer chip were initially homogeneously coated by PEGDA followed by exchange for an aqueous solution of the cell binding protein fibronectin and Bz. The solution was exposed by patterned UV light in the projection lithography setup using a 5× objective to project a checkerboard pattern onto the inner channel surface. After rinsing, a suspension of HT-29 colon cancer cells were added to the channels and incubated for

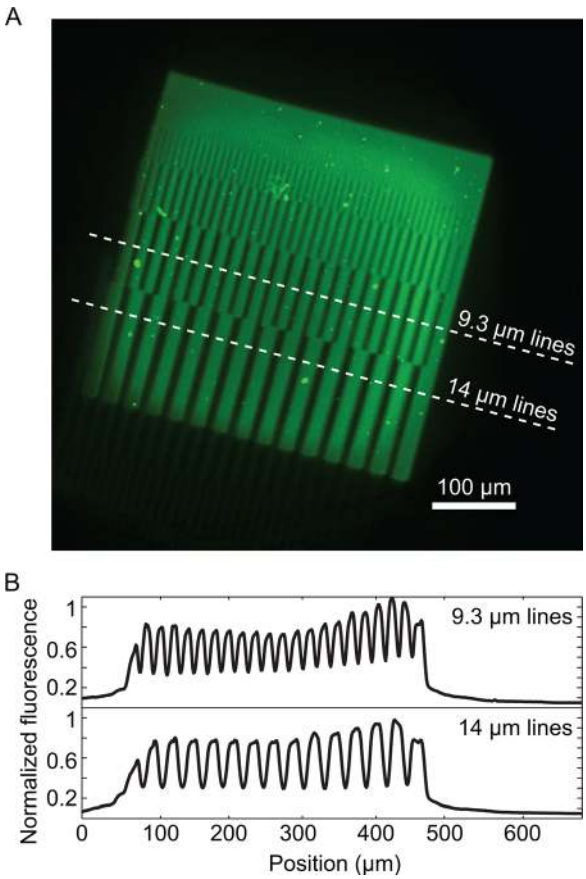

FIG. 5. (a) Fluorescence micrograph of fluorescein-BSA photo-immobilized on a PEGDA-coated surface inside the 70 μm high channel of a polyethylene microfluidic chip using projected patterned light exposure of a solution of fluorescein-BSA (100 μg/ml) and Bz (100 μg/ml). (b) Fluorescence intensity profiles along the dashed lines in (a) for mask motifs with nominal line-and-space distances of 9.3 μm and 14 μm, respectively. The channel surface was exposed using 365 nm light for 5 min at 720 mW/cm^2.

16 h before rinsing with PBS to remove unbound or loosely attached cells. Fibronectin was chosen as HT-29 is known to bind fibronectin through integrin receptors.[39] Figure 6 shows phase contrast micrographs of the surface patterned channel as well as unexposed reference channels. The HT-29 cells clearly adhered more strongly to the fibronectin patterned surface areas than the unexposed regions (Figure 6(a)). PEGDA coated channels without UV exposure during incubation with the fibronectin/Bz solution had few attached cells (Figure 6(b)), whereas complete cell coverage was observed in unexposed channels without a PEGDA coating to prevent non-specific adsorption of fibronectin (Figure 6(c)). HT-29 cells were also cultured for up to 72 h on the same layout of patterned fibronectin on PEGDA coating shown in Figure 6(a). Phase contrast micrographs recorded after 24 h and 72 h of culture (Figure S6 in Ref. 51) revealed cell proliferation on the fibronectin coated surface area at a rate indistinguishable from rates observed on tissue culture grade polystyrene (TCPS). Cell morphologies on photopatterned fibronectin were also indistinguishable from cells cultured on TCPS. Both observations suggest minimum leakage of cytotoxic compounds from the coatings.

IV. DISCUSSION

Covalent coating is generally preferred over a passively adsorbed coating as it ensures excellent stability and control over surface properties.[40] A number of methods for covalent modification in closed channel systems with reactive channel surfaces have been reported.[41] However, covalent coating on inert polymers like polystyrene or polyethylene usually requires

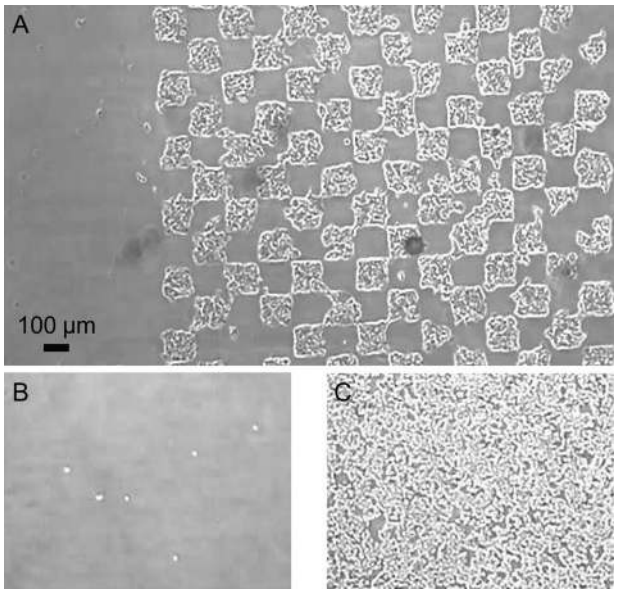

FIG. 6. Phase contrast micrographs of the adhesion of HT-29 colon cancer cells in microfluidic channels. (a) Cell adhesion on a PEGDA coated surface after patterned photo-immobilization of fibronectin. A solution of fibronectin (100 μg/ml) and Bz (1000 μg/ml) was exposed through the channel bottom for 20 min using a projection lithography system. The black spots are small air bubbles. (b) Cell adhesion on a PEGDA coated channel without UV exposure during incubation with fibronectin/Bz. (c) Cell adhesion in a channel without PEGDA coating and without UV exposure during incubation with fibronectin/Bz. The scale bar applies to all micrographs.

several harsh chemical activation steps to make the surface reactive towards the coating. These pre-steps can be difficult and time consuming to make, especially in a closed microfluidic system. Our method for coating the microfluidic channel in one-step resulted in a nanometer thin and covalently bound PEGDA coating. Other groups have made PEG acrylate coatings in microfluidic channels, and their results have highlighted some of the challenges.[11,14,32,42] Coatings could be made by adsorbing benzophenone dissolved in acetone on the channels prior to adding PEGDA and performing the photo-activation. The resulting layers were several micrometers thick, which is a problem in microfluidic channels containing micro-features that will be embedded. Moreover, the use of acetone as solvent may swell or dissolve the polymer channel walls.[11,14] Another approach is coating by suspension of the water insoluble benzophenone in an aqueous solution of PEG acrylate. Using this method, Luna-Vera *et al*. reported the formation of an uneven layer of globular PEG particles with an average thickness of 60 nm having the intended low protein binding properties.[42] However, Stachowiak *et al*. observed high protein binding for a similar PEG acrylate coating made with benzophenone suspended in aqueous buffer, which suggests that the suspension system may be highly sensitive to the exact experimental procedure.[32] The use of fully dissolved compounds to form a single homogeneous phase, as used in the current work, simplifies the process scheme and avoids potential issues with suspended and possibly aggregated particles. We have not explored the exact formation mechanism of the optimal thin PEGDA coating in great detail. However, our optimization results (Figure 2 and supplementary material Figs. S1 and S2 in Ref. 51) point to a balance between sufficiently dense activation (proton abstraction) of the polymer substrate surface at higher Bz concentrations, while minimizing detrimental proton abstraction from the formed PEGDA coating at even higher Bz concentrations. As briefly discussed in the supplementary material,[51] bulk PEGDA hydrogel formation will begin to occur upon increasing PEGDA concentrations 10-fold to above 50 mg/ml. However, for the low concentration of PEGDA used in this study (5 mg/ml), the rate of initiation in solution and the polymer volume fraction are too low to form a continuous bulk polymer network and any unbound PEGDA network fragments in solution will then be removed during subsequent washing. The temporal stability of

the resulting PEGDA coatings in a cell culture medium was indirectly validated for up to 3 days in longer term cell culture studies, where the HT-29 cells employed remained largely confined to the fibronectin-coated areas of the underlying PEGDA coating (Figure S6 in Ref. 51). In our former work using non-polymerizable PEG coatings, we did not observe any decrease in the coatings' ability to reduce protein adsorption after dry storage for more than a month.[37] The polymerized PEGDA coatings presented here are expected to exhibit equivalent temporal stability.

We have previously reported on the photopatterning of proteins with Bz onto a PEG-based surface coating in open microwell configurations.[37] Our current work significantly extends the range of applications of photoimmobilized low protein binding coatings by (a) demonstrating their *in-situ* one-step deposition in sealed thermoplastic microfluidic channel systems, (b) showing sufficient reduction in protein binding to prevent cellular attachment in reduced serum culture conditions, and (c) enabling the spatially selective attachment of cell adhesion promoting proteins in a local environment of sufficiently low cytotoxicity to allow for longer term cell culture. Spatially defined adhesion of cells onto a photo-immobilized protein pattern created inside a closed microfluidic channel has to our knowledge not been reported previously. Most patterning of cells in polymer microfluidic systems is performed by initial protein patterning on an open surface following by sealing of the channel system.[43,44] Cell adhesive patterns on open surfaces have been produced by numerous methods, including micro contact printing, physical masking, photolithography, and electrochemistry.[45–49] Maintaining the functionality of the proteins during channel sealing and possibly aligning the protein pattern to the channel geometry can be highly challenging. Many microfluidic systems fail due to high complexity in fabrication with limited flexibility in optimization and system integration.[50] Our method is easy to apply on almost any polymer surfaces as there is no requirement for specific functional groups other that the presence of carbons bound to hydrogen. Moreover, the coating and immobilization can be performed inside an already assembled microfluidic channel.

V. CONCLUSION

A nanometer thin PEGDA coating can be deposited from aqueous solution on open polymer surfaces and in closed polymer microchannels using a one-step photochemical procedure. The coating was demonstrated to limit both the non-specific adsorption of proteins and adhesion of mammalian cells. Moreover, protein in aqueous solution could be anchored onto the PEGDA coating in specific patterns within the closed polymer microchannels using an inverted microscope as a projection lithography system. A surface pattern of the cell binding protein fibronectin was shown to greatly improve cell adhesion compared to unexposed PEGDA-coated areas. These results pave the way for easy homogeneous or spatially defined coating of polymer surfaces and immobilization of protein in open or closed microsystems using only commercially available reagents.

ACKNOWLEDGMENTS

We thank Anne Henriksen for technical assistance in constructing the projection lithography setup. We acknowledge financial support from the Danish Advanced Technology Foundation through the PILOC project (Grant No. 064-2010-1) and the CELLGIGS project (Grant No. 25-2011-5), and from the Danish Council for Strategic Research through the IndiTreat project (Grant No. 10-092308).

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